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Adenosine A_{2A} receptors and depression

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Abstract—Adenosine and its analogues have been shown to induce "behavioral despair" in animal models believed to be relevant to depression. Recent data have shown that selective adenosine A_{2A} receptor antagonists (e.g., SCH 58261, ZM241385, and KW6002) or genetic inactivation of the receptor was effective in reversing signs of behavioral despair in the tail suspension and forced swim tests, two screening procedures predictive of antidepressant activity. A_{2A} antagonists were active in the tail suspension test using either mice previously screened for having high immobility scores or mice that were selectively bred for their spontaneous "helplessness" in this test. At stimulant doses, caffeine, a nonselective A₁/A_{2A} receptor antagonist, was effective in the forced swim test. The authors have hypothesized that the antidepressant-like effect of selective A_{2A} antagonists is linked to an interaction with dopaminergic transmission, possibly in the frontal cortex. In support of this idea, administration of the dopamine D₂ receptor antagonist haloperidol prevented antidepressant-like effects elicited by SCH 58261 in the forced swim test (putatively involving cortex), whereas it had no effect on stimulant motor effects of SCH 58261 (putatively linked to ventral striatum). The interaction profile of caffeine with haloperidol differed markedly from that of SCH 58261 in the forced swim and motor activity tests. Therefore, a clear-cut antidepressant-like effect could not be ascribed to caffeine. In conclusion, available data support the proposition that a selective blockade of the adenosine A_{2A} receptor may be an interesting target for the development of effective antidepressant agents.

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Adenosine, a multifaceted neuromodulator. It is well established that, in the CNS, adenosine is a neuromodulator acting through discrete cell-surface receptors.¹ Approximately 10 years ago, the first two adenosine receptors, A₁ and A_{2A}, were identified among putative G protein-coupled receptors. Later, two other receptor types, A_{2B} and A₃, were cloned.² Adenosine itself has been established as a potential regulator of complex central functions, such as anxiety states,^{3,4} aggressiveness,⁵ and sleep.⁶

Caffeine (1,3,7-trimethylxanthine) was recognized more than 20 years ago to act as an antagonist at adenosine receptors.⁷ Caffeine is likely to exert its primary action through adenosine receptors because they are the only known sites that bind caffeine at low concentrations.⁸ Caffeine has important effects on alertness and is widely consumed by people who need to stay awake.⁹ Stimulant effects have been quantified in motor activity studies in rodents.^{10,11}

Major depression is one of the most frequent illnesses, and increasing evidence suggests that it has a neurobiologic basis that includes genetic factors. Therefore, major depression may involve several neural systems within the brain, and the dopamine system is a candidate among them.^{12,13} Concerning the underlying functional neuroanatomy, it has been suggested that dysfunctions or imbalances at multiple points within limbic cortical-striatal-pallidal-thalamic circuits may be associated with major depressive syndrome.¹⁴

Adenosine and rodent's blues. Some experimental data suggest that adenosine may be involved in

the pathophysiology of mood disorders. First, the activation of central adenosine receptors, via either analogues of adenosine or an increase in endogenous adenosine levels, led to a behavioral state called "learned helplessness," similar to that induced by submitting rats to inescapable shocks.^{15,16} Second, in the mouse forced swim test,¹⁷ a preclinical test aimed at screening potential antidepressant agents, adenosine and its synthetic analogue 2-chloroadenosine lengthened the duration of immobility. Dipyrindamole, which is known to inhibit adenosine uptake, potentiated the adenosine effect. Conversely, the nonselective adenosine receptor antagonists caffeine and theophylline blocked the nucleoside-induced enhancement of immobility. In the same study, the tricyclic antidepressant agents imipramine and desipramine and the monoamine oxidase (MAO) inhibitor tranylcypromine also reversed adenosine-induced immobility.¹⁸ This prolongation of immobility in animals suggested that adenosine might be involved in the process leading to "behavioral despair."

The adenosine-dopamine connection put forward. Our understanding of adenosine-dopamine interactions in the basal ganglia^{19,20} indicates that adenosine modulates dopaminergic functions in dorsal and ventral striatum regions, where the nigrostriatal, the mesostriatal, and the mesolimbic dopaminergic neuronal pathways terminate. Adenosine A_{2A} receptors are predominantly expressed in pallidal-projecting GABAergic enkephalin-containing neurons, which also express dopamine D₂ receptors.^{5,21,22} Svenningsson et al.²³ have provided autora-

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diographic evidence for the existence of extrastriatal adenosine A_{2A} receptors throughout the thalamus and cerebral cortex in the human brain. Currently, there is interest in A_{2A} antagonists as therapeutic agents for dopamine-mediated motor disorders such as Parkinson's disease (PD).^{23,24} Conversely, increases in striatal dopamine D_2 receptor²⁵ and dopamine neuronal transporter²⁶ densities measured by SPECT have been reported in patients with depression as compared with control subjects. These effects may be associated with a reduction in dopaminergic function, reflected in either decreased dopamine release or dopamine receptor upregulation. The dopamine reuptake inhibitor bupropion²⁷ displays potent antidepressant properties.²⁸ Moreover, antidepressant effects in patients with depression have also been reported with direct dopamine D_2 agonists pibedil²⁹ and bromocriptine,³⁰ which are mainly used to manage PD. Therefore, adenosine receptor antagonists that modulate mesostriatal or mesocorticolimbic dopaminergic neuronal pathways may be therapeutically beneficial for the management of depression.

In addition to the mouse forced swim test,¹⁷ a second test called the automated tail suspension test³¹ is commonly used to screen potential antidepressant agents, which are considered effective if one dose can reduce the period of immobility in these tests. The mouse forced swim test does not regularly detect selective serotonin reuptake inhibitors,³² which are antidepressant agents. Therefore, for investigating antidepressant agents, it is interesting to add the tail suspension test to the mouse forced swim test because selective serotonin reuptake inhibitors are effective in the former.³³ One major drawback of these two tests is that they include a motor component unrelated to the correction of the pathologic disturbance of interest, i.e., low mood. Consequently, it is critical to carry out motor activity tests in parallel to try to differentiate pure motor stimulant effects from the ability to increase escape-directed behavior.^{37,38,34}

With this background in mind, it appeared logical to ask whether the blockade or absence of adenosine A_{2A} receptors would influence helplessness in animal models useful to screen potential antidepressant agents. Therefore, the behavior of A_{2A} receptor knockout ($A_{2A}R^{-/-}$) mice⁵ as compared with wild-type ($A_{2A}R^{+/+}$) controls has been evaluated in two different screening tests, and the effects of selective A_{2A} antagonists and caffeine on helplessness were also studied. Given their potentially confounding motor effects in these screening procedures, it appeared important to identify whether nonspecific changes in motor activity might be associated with any reversal of the state of despair.

Antidepressant-like behavioral effects in mice lacking the A_{2A} receptor. In the tail suspension test, the duration of immobility was significantly reduced in adenosine $A_{2A}R^{-/-}$ mice as compared with

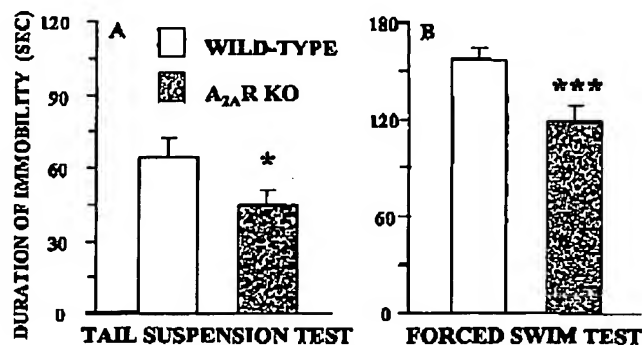


Figure 1. Immobility times of A_{2A} receptor knockout ($A_{2A}R^{-/-}$) and wild-type mice recorded in the tail suspension or forced swim tests. (A) Duration of immobility in the tail suspension test. Mean \pm SEM of data from 29 mice per group. (B) Duration of immobility in the forced swim test. Mean \pm SEM of data from 16 mice per group. * $p < 0.05$, *** $p < 0.001$ as compared with wild-type mice by Student's t-test. Reprinted from El Yacoubi et al.⁶⁰ Reproduced with permission.

$A_{2A}R^{+/+}$ animals. In the forced swim test, $A_{2A}R^{-/-}$ animals also behaved differently from their $A_{2A}R^{+/+}$ controls because they showed a strong reduction in the time of immobility (figure 1). Classically, these results may be interpreted as a reduction in helplessness in mice lacking the adenosine A_{2A} receptor. It was previously shown that adenosine $A_{2A}R^{-/-}$ mice displayed a reduced locomotor activity in an open field when compared with $A_{2A}R^{+/+}$ mice.^{5,11,35,36} On the contrary, in the forced swim and tail suspension tests, their activities were enhanced as compared with those of $A_{2A}R^{+/+}$ mice, suggesting that the neuronal pathways underlying the two behaviors are at least partly different. Furthermore, it is obvious that antidepressant-induced reduction of immobility cannot be explained by a nonspecific behavioral stimulation because many antidepressant agents tend to decrease motor activity.^{33,37} In addition, direct dopamine D_2 receptor agonists, known to usually reduce motor activity when administered in mice,³⁸ have been shown to increase mobility time in the forced swim test.³⁹ These encouraging results obtained with adenosine $A_{2A}R^{-/-}$ mice prompted the authors to study the effects of A_{2A} antagonists, nonselective and selective, with the expectation that they also have antidepressant-like properties.

Caffeine does not produce antidepressant-like effects in mice. Antidepressant-like properties of A_{2A} antagonists were initially suggested approximately 10 years ago by Sarges et al.⁴⁰ These authors discovered an antagonist compound, CP 66713, with a 25-fold selectivity toward A_{2A} vs A_1 receptor, which was active in the forced swim test. Caffeine, which at low doses causes "positive" subjective effects on mood,⁹ is a nonselective A_1 ($K_i = 29 \mu\text{mol/L}$) and A_{2A} ($K_i = 48 \mu\text{mol/L}$) antagonist.⁴¹ Functional studies revealed that the main targets for the stimulatory

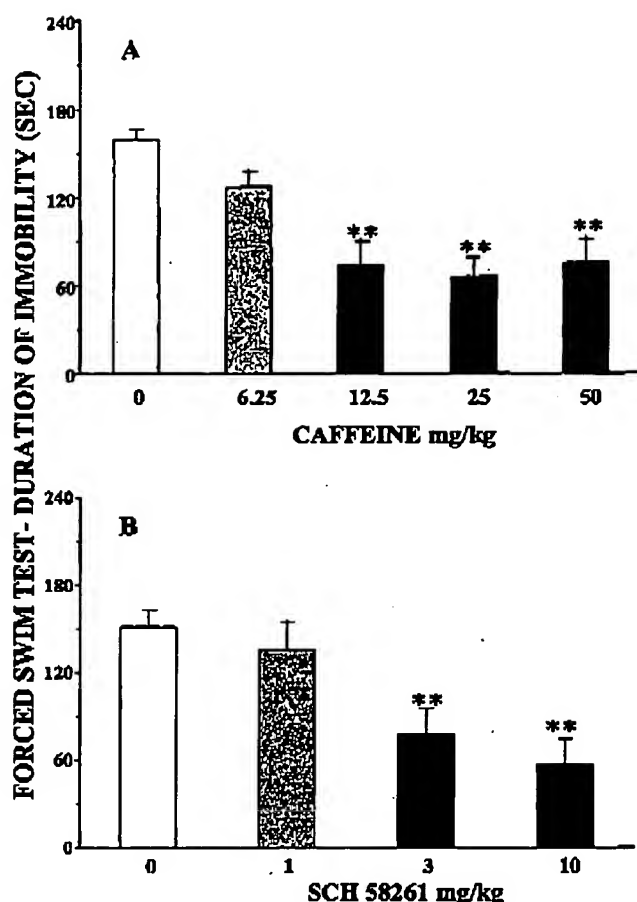


Figure 2. Effects of caffeine and SCH 58261 in the mouse forced swim test. Testing was for 6 minutes. (A) Caffeine (6.25, 12.5, 25, and 50 mg/kg) or (B) SCH 58261 (1, 3, and 10 mg/kg) were acutely administered intraperitoneally to CD1 mice. The test was performed 30 minutes after treatment. Mean \pm SEM of data from 8 to 14 control mice and 8 mice in treated groups. ** $p < 0.01$, (one-way analysis of variance followed by Student-Newman-Keuls test).

properties of caffeine⁷ are the adenosine A_{2A} receptors.^{10,11,43} A range of pharmacologically relevant doses of caffeine (6.25 to 50 mg/kg) administered to CD1 mice caused significant [$F(4,45) = 11.47$; $p < 0.001$] anti-immobility effects in the forced swim test (figure 2A), starting at the motor stimulant dose of 12.5 mg/kg.¹¹ These data are consistent with the results obtained in previous studies in mice.^{40,49} However, the fact that caffeine increases motor activity in mice (e.g., figure 4A) may cast doubt on the reliability of the forced swim test to identify this substance as a potential antidepressant. Therefore, to further analyze this effect of caffeine on the forced swim test, an interaction study with the preferential dopamine D₂ receptor antagonist haloperidol⁴⁴ was carried out. The aim was to discriminate an escape-directed behavior (i.e., a motivation to engage in active coping attempts to avoid the stressful situation) from a nonspecific locomotor stimulant effect elicited

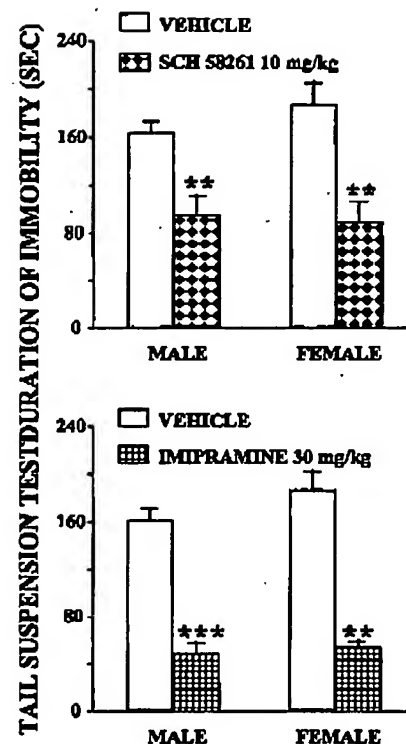


Figure 3. Effects of SCH 58261 or imipramine in the tail suspension test performed in a novel genetic mouse model of depression. Outbred CD1 mice were used as the foundation population of a line of mice that was selectively bred for its high spontaneous helplessness (immobility scores ≥ 115 seconds = helpless) in the tail suspension test. Mice of both sexes (seventh generation) were injected intraperitoneally with 10 mg/kg SCH 58261 (upper panel) or 30 mg/kg imipramine (lower panel) 30 minutes before the test. Testing was for 6 minutes. Mean \pm SEM of data from 8 to 12 control mice and 9 to 12 mice in treated groups. ** $p < 0.01$, *** $p < 0.001$ (one-way analysis of variance followed by Student-Newman-Keuls test) as compared with vehicle-injected groups.

by 25 mg/kg caffeine. Haloperidol depressed baseline and caffeine-stimulated open-field motor activity in a dose-dependent manner (see figure 4A). Two-way analysis of variance (ANOVA) showed that there was no haloperidol \times caffeine interaction [$F(2,47) = 2.05$; $p > 0.05$], indicating a parallel evolution of dose-response motor effects after haloperidol administration in vehicle- and caffeine-treated mice. The statistical analysis further showed significant effects of haloperidol and caffeine. By contrast, eticlopride, another preferential dopamine D₂ receptor antagonist, was shown to block the motor stimulant effects of caffeine in rats at doses not active on motor activity by themselves.⁴⁶ Finally, the anti-immobility effect of caffeine in the mouse forced swim test was prevented by pretreatment with haloperidol, as indicated by a significant interaction [$F(3,76) = 8.05$; $p < 0.001$] between haloperidol and caffeine treatments (figure 4B). Hence, the motor stimulant and

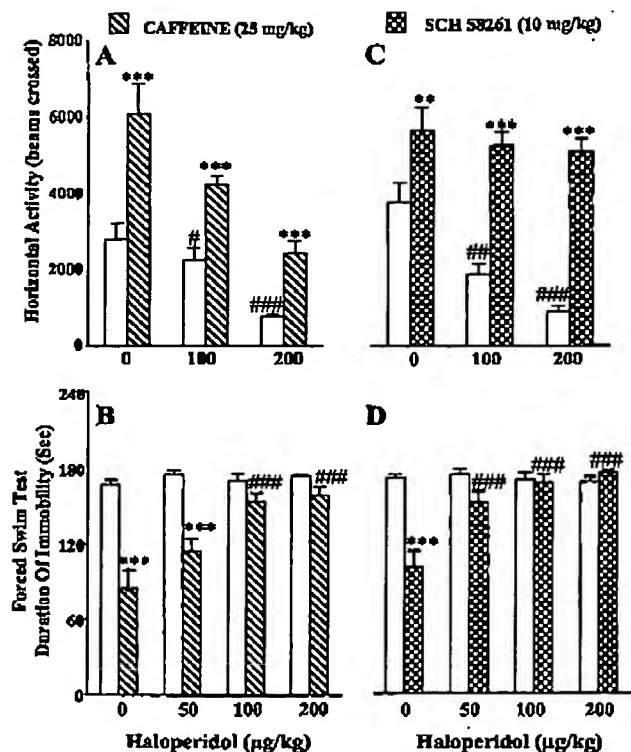


Figure 4. Effects of haloperidol on stimulation of locomotor activity and anti-immobility response induced by caffeine or SCH 58261. Mice were injected with saline or increasing doses of haloperidol (50, 100, and 200 µg/kg, intraperitoneally). Fifteen minutes later, they were injected intraperitoneally with vehicle or caffeine (25 mg/kg) or SCH 58261 (10 mg/kg). Upper panels, locomotor activity test. Immediately after the second treatment, mice were introduced into the actimeters. The horizontal activity was measured for 45 minutes. Mean \pm SEM of data from eight mice per group. Statistics by two-way analysis of variance (ANOVA) followed by post hoc comparisons. (A) Shows no interaction between factors haloperidol and caffeine: $F(2,47) = 2.05$, $p > 0.05$. (C) Reveals an interaction between factors haloperidol and SCH 58261: $F(2,47) = 4.11$, $p = 0.02$. Lower panels, forced swim test. Mice pretreated with haloperidol or saline received vehicle or caffeine or SCH 58261 30 minutes before testing. The duration of immobility was recorded during the last 3 minutes of the 6-minute testing period. Mean \pm SEM of data from 14 control mice and 8 to 11 mice in treated groups. Statistics by two-way ANOVA followed by post hoc comparisons. (B) Reveals an interaction between factors haloperidol and caffeine: $F(3,76) = 8.05$, $p < 0.001$. Also, (D) shows an interaction between factors haloperidol and SCH 58261: $F(3,72) = 5.04$, $p < 0.01$. Post hoc comparisons: ** $p < 0.01$, *** $p < 0.001$ as compared with respective caffeine- or SCH 58261-untreated control groups; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ as compared with respective haloperidol-untreated control groups. Panels C and D reprinted from El Yacoubi et al.⁵⁰ Reproduced with permission.

the anti-immobility effects of caffeine were altered by similar doses of the dopamine D₂ receptor antagonist haloperidol in the present work. These results suggest that caffeine could induce a nonspecific effect in the screening test used here. No genuine antidepressant effects have yet been attributed to caffeine despite the apparent increase it produces in norepinephrine turnover and the downregulation it induces in β -adrenoceptor levels.⁴⁶ Our results agree with other studies interpreting the effects of caffeine in the forced swim test as false positive.^{43,47} Given the effectiveness of adenosine A_{2A}R^{-/-} mice and of selective A_{2A} antagonists (see below) in the same screening procedures, we suggest that the antagonism at adenosine A₁ receptor elicited by caffeine could have negative effects on the behavioral outcome in the mouse forced swim test.

Selective A_{2A} antagonists are potential antidepressant agents. The effects of three selective A_{2A} antagonists, SCH 58261, ZM241385 and KW6002, have been examined in screening procedures for antidepressant agents. SCH 58261 is a selective adenosine A_{2A} receptor antagonist, with a greater selectivity profile than ZM241385.⁴⁸ The xanthine-like derivative KW6002 displays a high affinity for A_{2A} receptor, a moderate A_{2A} vs A₁ selectivity, and is active in experimental models of PD.⁴⁹

These three selective A_{2A} antagonists were shown to be effective in the tail suspension test after short-term treatment using CD1 mice.⁵⁰ The A_{2A} antagonists ZM241385 and SCH 58261 also decreased (by 50% for 30 mg/kg ZM241385, and by 68% for 10 mg/kg SCH 58261) the immobility time of animals screened twice as having a "high immobility" (i.e., immobility score ≥ 115 seconds) in the tail suspension test.⁵⁰ Finally, SCH 58261 and the tricyclic antidepressant imipramine increased struggling time in the latter test (figure 3) performed with mice that are selectively bred for their spontaneous helplessness in this test⁶⁰—a genetic mouse model that may be useful to screen potential antidepressant agents.⁶¹

Further experiments were carried out with the more selective compound SCH 58261, which does not target the adenosine A_{2B} receptor.⁴⁸ SCH 58261 was also found to be effective in the mouse forced swim test, with significant [$F(3,31) = 6.83$; $p = 0.001$] effects from 3 mg/kg, intraperitoneally (see figure 2B). Then, an interaction study with haloperidol was also carried out. In this set of two experiments performed in parallel, mice received increasing doses of subcutaneous haloperidol 15 minutes before the administration of an effective dose (10 mg/kg, intraperitoneally) of SCH 58261 and were then assayed in either the locomotor activity (figure 4C) or forced swim (figure 4D) tests. In the locomotor activity test, there was a significant haloperidol \times SCH 58261 interaction [$F(2,47) = 4.11$; $p < 0.05$]. As expected, haloperidol by itself induced a decrease in motor activity; however, the stimulant effects of SCH 58261 were not changed by the concomitant presence of haloperidol (see figure

4C). In the forced swim test, the two-way ANOVA also revealed a significant interaction between the two factors [$F(3,72) = 5.04$; $p < 0.01$]. Haloperidol produced no effect over the dose range used (see figure 4D). However, the effects of SCH 58261 in the forced swim test were reversed in the presence of haloperidol (50, 100, and 200 $\mu\text{g/kg}$, intraperitoneally). It is remarkable to note that the anti-immobility effect elicited by SCH 58261 was prevented by a low dose (0.05 mg/kg) of the dopamine D_2 receptor antagonist, demonstrating a high sensitivity of the goal-directed behavior to haloperidol (see figure 4D). This finding should be put into the context of other studies showing that dopamine D_2 receptor antagonists block anti-immobility effects of antidepressant agents.^{34,35} Haloperidol did not counteract SCH 58261-induced stimulant effects when administered in the range of doses used in the present experiment (see figure 4C). It may be pointed out that several previous studies have demonstrated that A_{2A} antagonists are effective in reducing catalepsy induced by high doses of dopamine D_2 receptor antagonists, a screening test for potential antiparkinsonian drugs.⁵³ Taken together, these results would fit with the hypothesis put forward by Svenningsson et al.⁴³ that adenosine and dopamine are tonically active at their respective receptors in striatum.

Therefore, dopamine transmission through D_2 receptors is critically involved in the anti-immobility effect elicited by SCH 58261. Dopamine transmission in the frontal cortex and nucleus accumbens has been implicated in the mechanism of action of antidepressant agents.^{43,54} One tentative explanation for the dissociation between the two behaviors studied here may reside in the peculiar physiology and pharmacology of dopamine neurons originating in the ventral tegmental area in the midbrain and projecting to the prefrontal cortex. As compared with mesolimbic and nigrostriatal dopamine neurons, mesocortical dopamine neurons have a higher firing rate, and dopamine has a higher turnover rate. Furthermore, mild stressors activate them. In contrast to nigrostriatal dopamine neurons, mesoprefrontal dopamine neurons also may not be well suited for maintaining homeostasis because of the absence or low sensitivity of synthesis- and impulse-regulating autoreceptors.⁵⁵ Because of the blockade of synthesis- and impulse-regulating autoreceptors on projections to dorsal and ventral striatum, haloperidol by itself may induce a greater release of dopamine and thus higher synaptic dopamine concentration in these areas, allowing a competition between haloperidol and synaptic dopamine. This could explain, in part, the lack of antagonism of SCH 58261-induced effects in the motor activity test. On the contrary, the dopamine release elicited by haloperidol in the frontal cortex would be much weaker in intensity,⁵⁶ allowing the reversal of the anti-immobility effect caused by the selective A_{2A} antagonist. Direct evidence of an increased extracellular dopamine release in the prefrontal cortex of rats, as reported for reference antidepressant agents such as

fluoxetine and clomipramine,⁵⁴ would lend support to our hypothesis.

The adenosine A_{2A} receptor has been visualized by autoradiography in the prefrontal cortex of the mouse⁴⁷ with densities equal to approximately one-tenth of that found in the striatum. The selective adenosine A_{2A} receptor antagonist [^3H] SCH 58261 was also found to label the postmortem human prefrontal cortex, with a binding density approximately one-third of that detected in rostral putamen.²² Numerous neuroanatomic studies have found differences in cerebral blood flow and metabolism in the prefrontal cortex of patients with depression when compared with healthy control subjects.¹⁴ Therefore, it will be of interest to investigate how A_{2A} receptor blockade produces an increase in escape-directed behavior in screening tests. A role of A_{2A} receptors located in the striatum cannot be completely excluded for at least two reasons. First, dopamine transmission in this structure plays an important role in determining the individual flexibility to manage sensory information.⁵⁸ Second, dopamine D_2 receptor densities are modified in the striatum of patients with depression relative to control subjects.²⁵

Although it is widely accepted that the tests discussed here are useful to screen potential antidepressant agents, selective A_{2A} antagonists should be screened in other preclinical models such as learned helplessness or chronic mild stress, for instance. Ultimately, the question of clinically significant antidepressant action in humans will be addressed when selective A_{2A} antagonists are evaluated in therapeutic trials for pathologic states such as PD²¹ if not for depression itself.

In conclusion, these data support the hypothesis that SCH 58261 and other A_{2A} antagonists induce activity in the forced swim and tail suspension tests by a prolongation of escape-directed behavior rather than by a generalized motor stimulant effect. The positive effect is likely mediated by an increase in dopaminergic transmission, possibly in the frontal cortex. Therefore, selective A_{2A} antagonists appear to be attractive targets for drug development as antidepressant agents.

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